

1. A non-naturally occurring or purified protein which inhibits human neutrophil elastase, and which is a protein comprising a mutant Kunitz domain, said domain being characterized by Cys at positions corresponding to bovine pancreatic trypsin inhibitor (BPTI) positions 5, 30, 51 and 55, Gly at a position corresponding to BPTI position 12, and Phe at a position corresponding to BPTI 33;

where the residues corresponding to BPTI positions 39-42 are not charged residues.

2. The protein of claim 1 in which the residues corresponding to BPTI positions 14 and 38 are Cys, and the residue corresponding to BPTI position 37 is Gly.

3. The protein of claim 2 in which the residue corresponding to BPTI position 45 is Phe and the residue corresponding to BPTI position 43 is Asn.

4. The protein of claim 1 where said mutant domain is at least 30% identical in amino acid sequence to BPTI.

5. The protein of claim 3 where said mutant domain is at least 30% identical in amino acid sequence to BPTI.

6. The protein of claim 1 where said mutant domain is at least 30% identical in amino acid sequence to ITI-D1.

7. The protein of claim 3 where said mutant domain is at least 30% identical in amino acid sequence to ITI-D1.

8. The protein of claim 2 where the residue corresponds to BPTI position 15 is Val, Ile or Leu.

9. The protein of claim 2 where the residue corresponding to BPTI position 15 is Val.

10. The protein of claim 2 where the residue corresponding to BPTI position 15 is Ile.

11. The protein of claim 2 where, the residue corresponding to BPTI position 15 is Leu.

12. The protein of claim 1 in which the residue corresponding to BPTI position 15 is Val and the residue corresponding to BPTI position 17 is Met.

13. The protein of claim 1 where the residue corresponding to BPTI position 15 is Leu, Ile or Val, the residue corresponding to BPTI position 16 is Ala or Gly, the residue corresponding to BPTI position 17 is Met, Phe, Ile or Leu, and the residue corresponding to BPTI position 19 is Pro, Ser, Lys or Gln.

14. The protein of claim 3 where the residue corresponding to BPTI position 15 is Leu, Ile or Val, the residue corresponding to BPTI position 16 is Ala or Gly, the residue corresponding to BPTI position 17 is Met, Phe, Ile or Leu, and the residue corresponding to BPTI position 19 is Pro, Ser, Lys or Gln.

15. The protein of claim 5 where the residue corresponding to BPTI position 15 is Leu, Ile or Val, the residue corresponding to BPTI position 16 is Ala or Gly, the residue corresponding to BPTI position 17 is Met, Phe, Ile or Leu, and the residue corresponding to BPTI position 19 is Pro, Ser, Lys or Gln.

16. The protein of claim 1 where the residue corresponding to BPTI position 16 is Ala.

17. The protein of claim 1 where the residue corresponding to BPTI position 17 is Phe.

18. The protein of claim 1 where the residue corresponding to BPTI position 19 is Pro.

19. The protein of claim 1 where the residues corresponding to BPTI position 39 is selected from the group consisting of Gly, Gln, Leu, Pro and Met.

20. The protein of claim 1 where the residue corresponding to BPTI position 39 is Met.

21. The protein of claim 1 where the residue corresponding to BPTI position 40 is Gly or Ala.

22. The protein of claim 1 in which the residue

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corresponding to BPTI position 40 is Gly.

23. The protein of claim 1 where the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp.

24. The protein of claim 1 in which the residue corresponding to BPTI position 41 is Asn.

25. The protein of claim 1 in which residue corresponding to BPTI position 42 is selected from the group consisting of Ala, Ser, Gly, Gln and Asn.

26. The protein of claim 1 in which the residue corresponding to BPTI position 42 is Gly.

27. The protein of claim 1

where the residue corresponding to BPTI position 39 is selected from the group consisting of Gly, Gln, Leu, Pro and Met,

where the residue corresponding to BPTI position 40 is Gly or Ala,

where the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp, and

where the residue corresponding to BPTI position 42 is selected from the group consisting of Ala, Ser, Gly, Gln and Asn.

28. The protein of claim 2

where the residue corresponding to BPTI position 39 is selected from the group consisting of Gly, Gln, Leu, Pro and Met,

where the residue corresponding to BPTI position 40 is Gly or Ala,

where the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp, and

where the residue corresponding to BPTI position 42 is selected from the group consisting of Ala, Ser, Gly, Gln and Asn.

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29. The protein of claim 3

where the residue corresponding to BPTI position 39 is selected from the group consisting of Gly, Gln, Leu, Pro and Met,

where the residue corresponding to BPTI position 40 is Gly or Ala,

where the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp, and

where the residue corresponding to BPTI position 42 is selected from the group consisting of Ala, Ser, Gly, Gln and Asn.

30. The protein of claim 5

where the residue corresponding to BPTI position 39 is selected from the group consisting of Gly, Gln, Leu, Pro and Met,

where the residue corresponding to BPTI position 40 is Gly or Ala,

where the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp, and

where the residue corresponding to BPTI position 42 is selected from the group consisting of Ala, Ser, Gly, Gln and Asn.

31. The protein of claim 13

where the residue corresponding to BPTI position 39 is selected from the group consisting of Gly, Gln, Leu, Pro and Met,

where the residue corresponding to BPTI position 40 is Gly or Ala,

where the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp, and

where the residue corresponding to BPTI position 42 is selected from the group consisting of Ala, Ser, Gly, Gln and

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corresponding to BPTI positions 39-42 are identical to the corresponding residues of a human Kunitz domain.

38. The protein of claim 13 where the residues corresponding to BPTI positions 39-42 are identical to the corresponding residues of a human Kunitz domain.

39. The protein of claim 14 where the residues corresponding to BPTI positions 39-42 are identical to the corresponding residues of a human Kunitz domain.

40. The protein of claim 15 where the residues corresponding to BPTI positions 39-42 are identical to the corresponding residues of a human Kunitz domain.

41. The protein of claim 27 where the residues corresponding to BPTI positions 39-42 are identical to the corresponding residues of a human Kunitz domain.

42. The protein of claim 33 where the residues corresponding to BPTI positions 39-42 are identical to the corresponding residues of a human Kunitz domain.

43. The protein of claim 1 where the residues corresponding to BPTI positions 40-42 are Gly-Asn-Gly.

44. The protein of claim 3 where the residues corresponding to BPTI positions 40-42 are Gly-Asn-Gly.

45. The protein of claim 5 where the residues corresponding to BPTI positions 40-42 are Gly-Asn-Gly.

46. The protein of claim 13 where the residues corresponding to BPTI positions 40-42 are Gly-Asn-Gly.

47. The protein of claim 14 where the residues corresponding to BPTI positions 40-42 are Gly-Asn-Gly.

48. The protein of claim 15 where the residues corresponding to BPTI positions 40-42 are Gly-Asn-Gly.

49. The protein of claim 13 where the residue corresponding to BPTI position 39 is Met.

50. The protein of claim 13 in which the residue corresponding to BPTI position 40 is Gly.

51. The protein of claim 13 in which the residue corresponding to BPTI position 41 is Asn.

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52. The protein of claim 13 in which the residue corresponding to BPTI position 42 is Gly.

53. The protein of claim 1 in which the residue corresponding to BPTI position 31 is selected from the group consisting of Gln, Glu, Leu, Lys, Val, Tyr and Asn.

54. The protein of claim 53 in which the residue corresponding to BPTI position 31 is Gln.

55. The protein of claim 1 in which the residue corresponding to BPTI position 34 is selected from the group consisting of Val, Ile, Thr, Asp, Asn, Gln, Phe, His, Pro, Arg and Lys.

56. The protein of claim 55 in which the residue corresponding to BPTI position 34 is Pro.

57. The protein of claim 1 in which the residue corresponding to BPTI position 31 is selected from the group consisting of Gln, Glu, Leu, Lys, Val, Tyr and Asn and the residue corresponding to BPTI position 34 is selected from the group consisting of Val, Ile, Thr, Asp, Asn, Gln, Phe, His, Pro, Arg and Lys.

58. The protein of claim 33 in which the residue corresponding to BPTI position 31 is selected from the group consisting of Gln, Glu, Leu, Lys, Val, Tyr and Asn and the residue corresponding to BPTI position 34 is selected from the group consisting of Val, Ile, Thr, Asp, Asn, Gln, Phe, His, Pro, Arg and Lys.

59. The protein of claims 40 in which the residue corresponding to BPTI position 31 is selected from the group consisting of Gln, Glu, Leu, Lys, Val, Tyr and Asn and the residue corresponding to BPTI position 34 is selected from the group consisting of Val, Ile, Thr, Asp, Asn, Gln, Phe, His, Pro, Arg and Lys.

60. The protein of claim 1 in which the residue corresponding to BPTI position 31 is Gln and which the residue corresponding to BPTI position 34 is Pro.

61. The protein of claim 33 in which the residue

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corresponding to BPTI position 31 is Gln and the residue corresponding to BPTI position 34 is Pro.

62. The protein of claim 40 in which the residue corresponding to BPTI position 31 is Gln and the residue corresponding to BPTI position 34 is Pro.

63. The protein of claim 1 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

64. The protein of claim 3 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

65. The protein of claim 5 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

66. The protein of claim 13 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

67. The protein of claim 14 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

68. The protein of claim 15 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

69. The protein of claim 27 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

70. The protein of claim 33 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

71. The protein of claim 34 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

72. The protein of claim 42 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

73. The protein of claim 57 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

74. The protein of claim 59 where, for each residue corresponding to a previously unspecified position in BPTI, the residue is one listed in Tables 15 or 34 as an amino acid found in the corresponding BPTI position in at least one of the BPTI homologues set forth in Table 13A.

75. The protein of claim 63 where, each such amino acid is one found in the corresponding BPTI position in an ITI Kunitz domain listed in Table 13A.

76. The protein of claim 63 where each amino acid is one found in the corresponding BPTI position in BPTI or human ITI-

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D1.

77. The protein of claim 1 where said domain has a higher percentage identity to a naturally-occurring human Kunitz domain of Table 13A than to any naturally-occurring nonhuman Kunitz domain of Table 13A.

78. The protein of claim 1 where said domain consists of an amino acid sequence identical to that of a protein selected from the group consisting of EpiNe α , EpiNE1, EpiNE2, EpiNE3, EpiNE4, EpiNE5, EpiNE6, EpiNE7, EpiNE8.

79. The protein of claim 1 where the amino acid sequence of said mutant Kunitz domain is of higher percentage identity to that of ITI-D1 than to that of BPTI.

80. The protein of claim 1 where the amino acid sequence of said mutant Kunitz domain is of higher percentage identity to that of BPTI than to that of ITI-D1.

81. The protein of claim 1 which has a binding affinity for human neutrophil elastase in the range of 100 pm to 1 pm.

82. The protein of claim 1 which has a binding affinity for human neutrophil elastase which is greater than that of BPTI (K1SV, R17L).

83. A non-naturally occurring or purified protein which inhibits human neutrophil elastase, and comprises a Kunitz domain whose amino acid sequence which at least 30% identical to that of BPTI,

where, in said sequence, the residue corresponding to BPTI position 18 is Phe, and

where the residues corresponding to BPTI positions 39-42 are not charged residues.

84. The protein of claim 2 where the residue corresponding to BPTI position 15 is Leu, Ile or Val, the residue corresponding to BPTI position 16 is Ala or Gly, the residue corresponding to BPTI position 17 is Met, Phe, Ile or Leu, and the residue corresponding to BPTI position 19 is Pro, Ser, Lys or Gln.

85. The protein of claim 4 where the residue

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corresponding to BPTI position 15 is Leu, Ile or Val, the residue corresponding to BPTI position 16 is Ala or Gly, the residue corresponding to BPTI position 17 is Met, Phe, Ile or Leu, and the residue corresponding to BPTI position 19 is Pro, Ser, Lys or Gln.

86. The protein of claim 3 where the residue corresponds to BPTI position 15 is Val, Ile or Leu, the residue corresponding to BPTI position 16 is Ala, the residue corresponding to BPTI position 17 is Phe, and the residue corresponding to BPTI position 19 is Pro.

87. The protein of claim 4 where the residue corresponds to BPTI position 15 is Val, Ile or Leu, the residue corresponding to BPTI position 16 is Ala, the residue corresponding to BPTI position 17 is Phe, and the residue corresponding to BPTI position 19 is Pro.

88. The protein of claim 5 where the residue corresponds to BPTI position 15 is Val, Ile or Leu, the residue corresponding to BPTI position 16 is Ala, the residue corresponding to BPTI position 17 is Phe, and where the residue corresponding to BPTI position 19 is Pro.

89. The protein of claim 2 where the residue corresponding to BPTI position 39 is selected from the group consisting of Arg, Gly, Lys, Gln, Asp, Glu, Leu, Pro and Met,

the residue corresponding to BPTI position 40 is Gly or Ala,

the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp, and

the residue corresponding to BPTI position 42 is selected from the group consisting of Ala, Ser, Gly, Gln and Asn.

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the residue corresponding to BPTI position 39 is selected from the group consisting of Arg, Gly, Lys, Gln, Asp, Glu, Leu, Pro and Met.

the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp, and

91. The protein of claim 6 where

the residue corresponding to BPTI position 40 is Gly or Ala,

the residue corresponding to BPTI position 42 is selected from the group consisting of Ala, Ser, Gly, Gln and Asn.

the residue corresponding to BPTI position 39 is selected from the group consisting of Arg, Gly, Lys, Gln, Asp, Glu, Leu, Pro and Met,

the residue corresponding to BPTI position 41 is selected from the group consisting of Asn, Gln, Ser, Thr, Tyr and Trp, and

93. The protein of claim 33 where

the residue corresponds to BPTI position 15 is Val, Ile

or Leu the residue corresponding to BPTI position 16 is Ala,
the residue corresponding to BPTI position 17 is Phe, and
the residue corresponding to BPTI position 19 is Pro.

94. The protein of claim 93 where
the residue corresponding to BPTI position 31 is selected
from the group consisting of Gln, Glu, Leu, Lys, Val, Tyr and
Asn, and

the residue corresponding to BPTI position 34 is selected
from the group consisting of Val, Ile, Thr, Asp, Asn, Gln,
Phe, His, Pro, Arg and Lys.

95. The protein of claim 94 where the residue
corresponding to BPTI position 31 is Gln and the residue
corresponding to BPTI position 34 is Pro.

96. A method of inhibiting human neutrophil elastase
which comprises contacting the elastase with the protein of
claims 1, 2, 3, 4, 5, 13, 27, 29, 30, 83, or 94.

97. A method of inhibiting harmful human neutrophil
elastase activity in a subject which comprises administering
to the subject an inhibitorily effective amount of the protein
of any of claims 1-95.

98. A method of treating emphysema which comprising
administering, to a subject suffering from emphysema, a
therapeutically effective amount of the protein of claims 1,
2, 3, 4, 5, 13, 27, 29, 30, 83 or 94.

99. A method of treating cystic fibrosis which
comprising administering, to a subject suffering from cystic
fibrosis, a therapeutically effective amount of the protein of
claims 1, 2, 3, 4, 5, 13, 27, 29, 30, 83 or 94.

100. A method of inhibiting harmful human neutrophil
elastase activity in a subject which comprises administering
to the subject an inhibitorily effective amount of the protein
of any of claims 1, 2, 3, 4, 5, 13, 27, 29, 30, 83 or 94,

where the Kd of said protein for trypsin, chymotrypsin
and plasma is greater than 10^{-6} M.